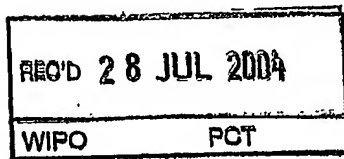




PCT/EP200 4 / 0 107 4 3 7



INVESTOR IN PEOPLE



The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., pl. P.L.C. or PLC.

Registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated

08 APR 2004

BEST AVAILABLE COPY

**PRIORITY
DOCUMENT**
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

08 JUL 2003

LONDON

The
Patent
Office

1/77
09JUL03 E821075-6 D00245
P01/7700 0.00-0315965.4

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales NP10 8QQ

1.	Your reference	4-33258P1		
2.	Patent application number (The Patent Office will fill in this pc .	0315965.4		
3.	Full name, address and postcode of the or of each applicant (underline all surnames)	NOVARTIS AG LICHTSTRASSE 35 4056 BASEL SWITZERLAND		
	Patent ADP number (if you know it)			
	If the applicant is a corporate body, give the country/state of its incorporation	SWITZERLAND		
4.	Title of invention	7125487005 Organic compounds		
5.	Name of your agent (If you have one) "Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)	Craig McLean Novartis Pharmaceuticals UK Limited Patents and Trademarks Wimblehurst Road Horsham, West Sussex RH12 5AB		
	Patents ADP number (if you know it)	7181522002		
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number	Country	Priority application number (if you know it)	Date of filing (day/month/year)
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing (day/month/year)	
8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body. (see note (d))	Yes		

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form

Description 7

Claim(s) 2

Abstract

Drawing(s)

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (*Patents Form 7/77*)

Request for preliminary examination and search (*Patents Form 9/77*) One

Request for substantive examination (*Patents Form 10/77*)

Any other documents
(please specify)

11. I/We request the grant of a patent on the basis of this application

Signature

Date

8th July 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

Mrs. S. Schnerr

01403 323069

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.
- Write your answers in capital letters using black ink or you may type them.
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- Once you have filled in the form you must remember to sign and date it.
- For details of the fee and ways to pay please contact the Patent Office.

DUPLICATE

- 1 -

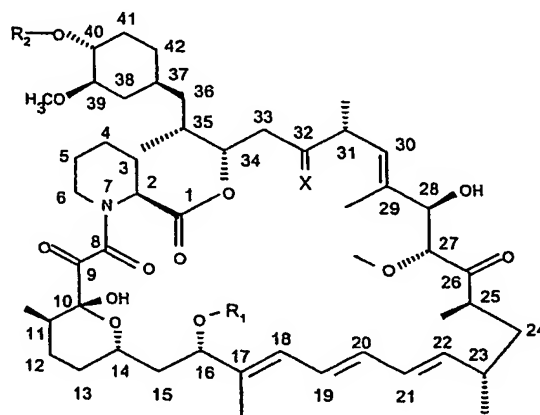
Organic Compounds

The present invention relates to a new use of rapamycin and rapamycin derivatives.

Rapamycin is an immunosuppressive lactam macrolide that is produced by Streptomyces hygroscopicus.

A rapamycin derivative is a substituted rapamycin e.g. a 40-O-substituted rapamycin e.g. as described in US 5 258 389, WO 94/09010, WO 92/05179, US 5 118 677, US 5 118 678, US 5 100 883, US 5 151 413, US 5 120 842, WO 93/11130, WO 94/02136, WO 94/02485 and WO 95/14023 all of which are incorporated herein by reference; a 16-O-substituted rapamycin e.g. as disclosed in WO 94/02136, WO 95/16691 and WO 96/41807, the contents of which are incorporated herein by reference; or a 32-hydrogenated rapamycin e.g. as described in WO 96/41807 and US 5 256 790, incorporated herein by reference.

Preferred rapamycin derivatives are compounds of formula I



wherein

R_1 is CH_3 or C_{3-6} alkynyl,

R_2 is H or $-\text{CH}_2-\text{CH}_2-\text{OH}$, 3-hydroxy-2-(hydroxymethyl)-2-methyl-propanoyl or tetrazolyl, and X is $=\text{O}$, (H,H) or (H,OH)

provided that R_2 is other than H when X is $=\text{O}$ and R_1 is CH_3 ,

or a prodrug thereof when R_2 is $-\text{CH}_2-\text{CH}_2-\text{OH}$, e.g. a physiologically hydrolysable ether thereof.

Particularly preferred rapamycin derivatives of formula I are 40-O-(2-hydroxyethyl)-rapamycin (Compound A hereinafter), 40-[3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate]-rapamycin (also called CCI779), 40-epi-(tetrazolyl)-rapamycin (also called

ABT578), 32-deoxorapamycin, 16-pent-2-ynyloxy-32(S)-dihydro rapamycin, or TAFA-93. Even more preferred is Compound A.

Rapamycin derivatives also include so-called rapalogs, e.g. as disclosed in WO 98/02441 and WO 01/14387, e.g. AP23573, AP23464, or AP23841.

Rapamycin and derivatives thereof have, on the basis of observed activity, e.g. binding to macrophilin-12 (also known as FK-506 binding protein or FKBP-12), e.g. as described in WO 94/09010, WO 95/16691 or WO 96/41807, been found to be useful e.g. as immuno-suppressant, e.g. in the treatment of acute allograft rejection.

It has now been found that rapamycin and derivatives thereof are useful for the treatment of abnormally increased bone turnover or resorption.

In accordance with the particular findings of the present invention, there is provided:

1. A method for treating abnormally increased bone turnover or resorption in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a rapamycin derivative.

In particular, there is provided:

- 1.1 A method for treating osteoporosis, e.g. postmenopausal osteoporosis, postmenopausal bone loss; male osteoporosis; corticosteroid-induced osteoporosis, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a rapamycin derivative.
- 1.2 A method for treating bone loss secondary to or due to medication, e.g. diphenylhydantoin, thyroid hormone replacement therapy; in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a rapamycin derivative.
- 1.3 A method for treating bone loss associated with immobilisation and space flight; in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a rapamycin derivative.
- 1.4 A method for treating bone loss associated with rheumatoid arthritis, osteopenia, osteogenesis imperfecta, hyperthyroidism, anorexia nervosa, organ transplantation, joint prosthesis loosening, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a rapamycin derivative.

- 1.5 A method for treating periarticular bone erosions in rheumatoid arthritis, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a rapamycin derivative.
- 1.6 A method for treating osteoarthritis, e.g. subchondral osteosclerosis, subchondral bone cysts, osteophyte formation, and of osteoarthritic pain, e.g. by reduction in intra-osseous pressure, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a rapamycin derivative.
- 1.7 A method for treating hypercalcemia, e.g. tumour-induced hypercalcemia, e.g. resulting from excessive bone resorption secondary to hyperparathyroidism, thyrotoxicosis, sarcoidosis or hypervitaminosis D, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a rapamycin derivative.
- 1.8 A method for treating bone cancer and bone metastases, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a rapamycin derivative; in particular a method for treating bone cancer and bone metastases induced by a primary tumour, e.g. breast or prostate cancer.
- 1.9 A method for treating multiple myeloma, in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a rapamycin derivative.

In the present description the terms "treatment" or "treat" refer to both prophylactic or preventive treatment as well as curative or disease modifying treatment, including treatment of patients at risk of contracting the disease or suspected to have contracted the disease as well as patients who are ill or have been diagnosed as suffering from a disease or medical condition.

In a series of further specific or alternative embodiments, the present invention also provides:

2. Rapamycin or a rapamycin derivative for use in any method as defined under 1, in particular under 1.1 to 1.9 above.
3. Rapamycin or a rapamycin derivative for use in the preparation of a pharmaceutical composition for use in any method as defined under 1, in particular under 1.1 to 1.9 above.

4. A pharmaceutical composition for use in any method as defined under 1, in particular under 1.1 to 1.9 above, comprising rapamycin or a rapamycin derivative together with one or more pharmaceutically acceptable diluents or carriers therefore.

Rapamycin or a rapamycin derivative may be administered as the sole drug or in combination with a second drug. Suitable drugs for combination include a bone resorption inhibitor, e.g. as in osteoporosis therapy, in particular a calcitonin or an analogue or derivative thereof, e.g. salmon, eel or human calcitonin; a steroid hormone, e.g. an estrogen, a partial estrogen agonist or estrogen-gestagen combination; a selective estrogen receptor modulator (SERM) e.g. raloxifene, lasofoxifene, TSE-424, FC1271; tibolone (Livial ®); vitamin D or an analogue thereof; Parathyroid Hormone (PTH), a PTH fragment or a PTH derivative e.g. PTH (1-84), PTH (1-34), PTH (1-36), PTH (1-38), PTH (1-31)NH₂ or PTS 893; a bisphosphonate e.g. alendronate, zoledronic acid, ibandronate; a cathepsin K inhibitor; PTH releaser; a selective androgen receptor molecule (SARM); metalloprotease (MMP) inhibitor; or strontium ranelate.

Accordingly, in another aspect, the present invention provides:

5. A pharmaceutical combination comprising a) rapamycin or a rapamycin derivative, and b) a second drug, e.g. as exemplified above.
6. A method as defined above comprising co-administration, e.g. concomitantly or in sequence, of a therapeutically effective amount of rapamycin or a rapamycin derivative, and a second drug, e.g. as exemplified above.

The terms "co-administration" or "combined administration" or the like as utilized herein are meant to encompass administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the drugs are administered together, one after the other or separately in one combined unit dosage form or in two separate unit dosage forms but also in which the drugs are not necessarily administered by the same route of administration or at the same time. A unit dosage form may also be a fixed combination.

Utility of the compounds of the invention in treating diseases and conditions as hereinabove specified, may be demonstrated in standard animal or clinical tests, e.g. in accordance with the methods described hereinafter.

A. In vitro

A.1 Mouse Osteoclastogenesis Assay

Non-adherent bone marrow mononuclear cells from 5-week-old male mice cells are differentiated into bone-resorbing osteoclasts by treatment with a cytokine cocktail containing receptor activator of NF kappa B ligand (RANKL), macrophage-colony stimulating factor (M-CSF) and interleukin-1 (IL-1) alpha. Osteoclast formation is measured after 6 days by quantifying the number of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells generated in plastic wells on a 48-well plate. Osteoclast activity is measured after 12 days by quantifying the area of resorbed dentine slices placed in wells on a 48-well plate. Treatment with rapamycin or the rapamycin derivative, e.g. Compound A, starts at the beginning of cell culture, together with the cytokine treatment.

Osteoblast differentiation is evaluated in mouse pre-osteoblastic cell line MC3T3-1b, stimulated to differentiate with osteogenic stimulus (a mixture of bone morphogenetic protein 2 (BMP-2), ascorbic acid and beta-glycerophosphate). Osteoplast activity is measured by quantifying culture area covered with alkaline phosphate-positive cells on a 48-well plate. Treatment with rapamycin or the rapamycin derivative, e.g. Compound A, starts at the beginning of cell culture, together with the osteogenic stimulus treatment.

In this assay, rapamycin or the rapamycin derivatives inhibit osteoclast formation and activity at an $IC_{50} < 1\mu\text{m}$.

Using Compound A, osteoclast formation is inhibited with an IC_{50} of 10.5 ± 4.6 nM and osteoclast activity with an IC_{50} of 0.6 ± 0.3 nM for osteoclast activity. Alkaline phosphatase (ALP) staining has an IC_{50} of 13.5 ± 2.4 nM.

A.2 Human Osteoclastogenesis Assay

Peripheral blood mononuclear cells from healthy male donors are differentiated into bone-resorbing osteoclasts by treatment with a cytokine cocktail containing RANKL, M-CSF and transforming growth factor (TGF)-beta 1. Osteoclast formation is measured after 17 days by quantifying the number of TRAP-positive multinucleated cells generated in plastic wells on a 96-well plate. Osteoclast activity is measured after 17 days by quantifying the area of resorbed bone on bovine cortical bone slices placed in wells on a 96-well plate. Treatment with rapamycin or the rapamycin derivative, e.g. Compound A, starts at the beginning of cell culture, together with the cytokine treatment. Collagen fragments are measured by enzyme linked immunosorbent assay (ELISA).

In this assay, rapamycin or the rapamycin derivatives inhibit osteoclast formation at an $IC_{50} < 1 \mu M$.

Using Compound A, osteoclast formation is inhibited with an IC_{50} values of 7.7 ± 1.1 nM. Resorbed area is inhibited with an IC_{50} of 3.4 ± 0.3 nM. Collagen fragment release is inhibited with an IC_{50} of 4.0 ± 0.5 nM.

Rapamycin and rapamycin derivatives are evaluated for in vivo bone resorption inhibition in an animal model e.g. as disclosed in Shinoda et al., *Calcif. Tissue Int.*, 1983, 35, 87-99 or Schenk et al. *Calcif. Tissue Res.* 1973, 11, 196-214, or e.g. as disclosed hereinafter.

B. In vivo: Ovariectomized rat model

Before operation, the tibial bone mass and geometry of the animals is measured at baseline by dual-energy x-ray absorptiometry (DEXA) and peripheral quantitative computer tomography (pQCT). Following ovariectomy (OVX) or sham operation, skeletally mature rats are treated for 8 weeks daily with 0.15 mg/kg, 0.5 mg/kg, 1.5 mg/kg, or 3.0 mg/kg of rapamycin or a rapamycin derivative, e.g. Compound A, or vehicle alone by oral administration or once a week with 1.5 mg/kg or 5.0 mg/kg of rapamycin or a rapamycin derivative, e.g. Compound A. At the beginning of the treatment, animals receive a fluorochrome label, e.g. calcein (e.g. 30mg/kg, subcutaneous (s.c.)). Changes in bone mass and geometry (pQCT, DEXA) are evaluated in vivo after 4 weeks of treatment and at 8 weeks before necropsy. Body weight is monitored weekly. The animals are administered two further fluorochrome labels for marking of bone mineralization prior to necropsy, e.g. alizarin e.g. 20mg/kg, s.c., 10 days prior to necropsy, and calcein e.g. 30mg/kg, s.c., 3 days prior to necropsy. Blood samples (500 μ l blood) are taken in heparin before necropsy and frozen at -20°C for analysis of calcium, phosphate, TRAP, ALP, and osteocalcin. DEXA measurements are carried out at necropsy on excised tibia, femur, and lumbar vertebrae.

For example, Compound A reduces cancellous bone loss with 60% inhibition at 3 mg/kg/day, and inhibits reduction of trabecular number.

Daily dosages required in practicing the method of the present invention when rapamycin or a rapamycin derivative alone is used will vary depending upon, for example, the compound used, the host, the mode of administration and the severity of the condition to be treated. A preferred daily dosage range is about from 0.1 to 25 mg as a single dose or in divided doses. Suitable daily dosages for patients are on the order of from e.g. 0.1 to 25 mg p.o. Rapamycin or a rapamycin derivative may be administered by any conventional route, in

particular enterally, e.g. orally, e.g. in the form of tablets, capsules, drink solutions, nasally, pulmonary (by inhalation) or parenterally, e.g. in the form of injectable solutions or suspensions. Suitable unit dosage forms for oral administration comprise from ca. 0.05 to 12.5 mg, usually 0.25 to 10 mg of rapamycin or a rapamycin derivative, e.g. Compound A, together with one or more pharmaceutically acceptable diluents or carriers therefor.

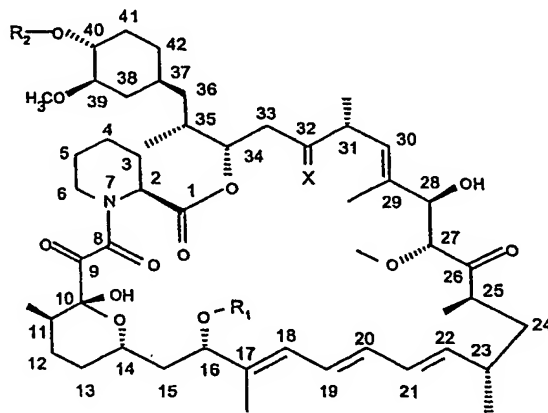
Due to synergism lower doses of the drugs of the combination of the invention may be used, for example, the dosages need not only often be smaller but are also applied less frequently, or may be used in order to diminish the incidence of side-effects. This is in accordance with the desires and requirements of the patients to be treated.

When rapamycin or the rapamycin derivative is co-administered with a second drug, dosages for the co-administered drug will of course vary depending on the type of drug employed, e.g. whether it is a steroid, a calcitonin or a biphosphonate, on the specific drug employed, on the condition to be treated, the severity of the condition being treated, whether it is a curative or preventive therapy, on the regimen and so forth.

The pharmaceutical compositions for separate administration of rapamycin or a rapamycin derivative and a second drug and for the administration in a fixed combination, i.e. a single galenical composition comprising at least two combination partners, according to the invention may be prepared in a manner known per se comprising a therapeutically effective amount of at least one pharmacologically active combination partner alone, e.g. as indicated above, or in combination with one or more pharmaceutically acceptable carriers or diluents.

CLAIMS

1. Use of rapamycin or a rapamycin derivative in the preparation of a pharmaceutical composition for the treatment of abnormally increased bone turnover or resorption.
2. A pharmaceutical composition for use in the treatment of abnormally increased bone turnover or resorption comprising rapamycin or a rapamycin derivative, together with one or more pharmaceutically acceptable diluents or carriers therefor.
3. A pharmaceutical combination comprising rapamycin or a rapamycin derivative and a second drug selected from bone resorption inhibitor, a calcitonin or an analogue or derivative thereof; a steroid hormone, a partial estrogen agonist or estrogen-gestagen combination; a selective estrogen receptor modulator; vitamin D or an analogue thereof; Parathyroid Hormone (PTH), a PTH fragment or a PTH derivative; a bisphosphonate; a cathepsin K inhibitor; a PTH releaser; a selective androgen receptor molecule; a metalloprotease inhibitor; and strontium ranelate.
4. A method for treating abnormally increased bone turnover or resorption in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of rapamycin or a rapamycin derivative, optionally concomitantly or sequentially with a chemotherapeutic agent.
5. Use, combination or method according to any preceding claim wherein the rapamycin derivative is a compound of formula I



wherein

R₁ is CH₃ or C₃₋₆alkynyl,

R₂ is H or -CH₂-CH₂-OH, 3-hydroxy-2-(hydroxymethyl)-2-methyl-propanoyl or tetrazolyl,

and X is =O, (H,H) or (H,OH),

provided that R_2 is other than H when X is =O and R_1 is CH_3 ,
or a prodrug thereof when R_2 is $-CH_2-CH_2-OH$, e.g. a physiologically hydrolysable
ether thereof.

6. Use, a method, or a combination substantially as hereinbefore disclosed or defined.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.